Genome stability: **Failure to unwind causes cancer**

Paul M. Watt and Ian D. Hickson

The recent cloning of the gene defective in individuals with Bloom's syndrome has revealed a link between DNA helicases, genetic instability and a predisposition to cancer.

Address: Imperial Cancer Research Fund Laboratories, Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK.

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Several rare human genetic diseases are characterized at the cellular level by a high incidence of chromosomal rearrangements. Some of these disorders — in particular, xeroderma pigmentosum — are associated with abnormalities in the repair of DNA damage, whereas others — notably Fanconi's anaemia and Bloom's syndrome — are not. Rapid progress has been made in recent years in the characterisation of the genetic basis for these abnormalities. The recent cloning of the gene, *BLM*, that is defective in Bloom's syndrome has opened the way to a detailed analysis of the BLM protein and to an understanding of the biochemical basis underlying one cause of genomic instability.

Bloom's syndrome individuals exhibit a number of abnormalities, including pre-natal and post-natal growth retardation, immunodeficiency and a greatly increased incidence of cancer. Indeed, of the human syndromes characterized by genomic instability, Bloom's syndrome is associated with the highest incidence of malignant neoplasms, with the mean age at cancer diagnosis among affected individuals being only 25 years. Moreover, in contrast to all other genome instability disorders, Bloom's syndrome is associated with an increased incidence of many different cancers, including both leukaemias and solid tumours. This suggests that defects in *BLM* influence the proper functioning of all cell types in the body.

A wide body of evidence has accumulated indicating that the cytogenetic abnormalities displayed by Bloom's syndrome cells result from an increased incidence of chromosomal rearrangements between homologous sequences. Although these rearrangements take many different forms, a hallmark of the disease is a 10–20-fold elevation in the frequency of spontaneous sister-chromatid exchanges (reviewed in [1]). Furthermore, the high incidence of symmetrical, quadriradial chromosomes — presumed to be homologous chromosomes caught in the act of recombination — in Bloom's syndrome cells is indicative of an elevated frequency of crossing-over between homologous chromosomes.

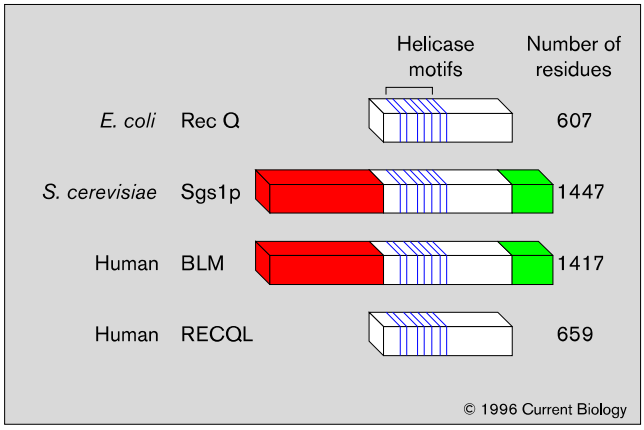
The cloning of the *BLM* gene is the culmination of many years' work on Bloom's syndrome in the laboratory of James German [2]. The elegant cloning strategy that German and colleagues adopted, which exploited the high incidence of mitotic hyper-recombination events in Bloom's syndrome cells, has been reviewed recently [3]. We shall focus on the potential roles of the *BLM* gene product, with reference to knowledge that has been gained from studies on bacterial and lower eukaryotic homologues of *BLM*.

The *BLM* gene is predicted to encode a 159 kDa protein with a number of identifiable structural motifs (Fig. 1). In particular, the protein includes seven domains that are conserved in a variety of ATP-dependent DNA and RNA helicase enzymes. The BLM protein is most strongly related to a helicase family of which the prototypical member is the *Escherichia coli* RecQ protein [4,5]. This family also includes the *Saccharomyces cerevisiae* Sgs1 protein [6,7] and the human RECQL protein (also known as helicase Q1) [8,9]. Two of these proteins have been shown to be *bona fide* DNA helicases, and it is therefore likely that the BLM protein is also a helicase. Within the region encompassing the seven helicase domains, the BLM protein shows 42–44 % sequence identity with RecQ, Sgs1p and RECQL.

There is, however, considerable structural and sequence divergence between these proteins outside of the helicase domains. Most strikingly, BLM and Sgs1p share a serine rich, highly charged amino-terminal domain of some 575 amino acids, which is completely absent from both RecQ and RECQL (Fig. 1). Thus, in structural terms, it is Sgs1p that most closely resembles BLM, although their amino-terminal domains show only weak sequence similarity. Whether this indicates that the RecQ family of helicases can be separated into two functional sub-groups, based upon the presence or absence of this extensive amino-terminal domain, remains to be determined.

The *SGS1* gene was isolated by two independent strategies, although both were designed to investigate the function of topoisomerases. In one case, *sgs1* was identified as a suppressor of the slow-growth phenotype of *top3* mutants, which are deficient in topoisomerase III, and

Figure 1



Schematic representation of the domain structure of BLM and its homologues. The number of amino acid residues in each protein is indicated on the right. The amino-terminal and carboxy-terminal domains found only in Sgs1p and BLM are shown in red and green, respectively. The blue vertical lines denote the seven helicase domains, designated from left to right: I, Ia, II, III, IV, V and VI. Domain I contains the consensus ATP-binding motif; GXGKS/T. The RecQ family of helicases is characterised by having a DEXH motif in helicase domain II.

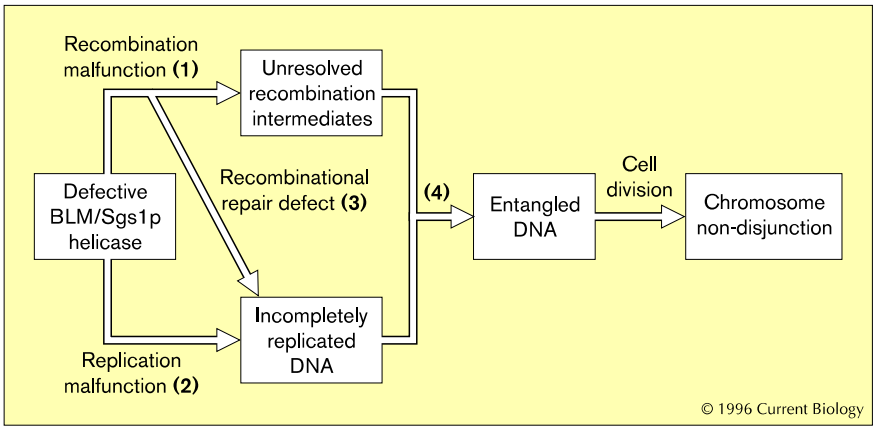
Sgs1p was shown to interact with topoisomerase III in yeast [6]. Topoisomerase III is a poorly characterized type I topoisomerase — that is, it acts *via* the formation of single-stranded breaks — implicated in suppression of excessive genetic recombination. In the second strategy, Sgs1p was identified as a protein that binds topoisomerase II using the two-hybrid cloning system [7]. Topoisomerase II is an essential enzyme that acts to disentangle intertwined DNA, and is required for chromosome segregation during cell division (reviewed in [10]). Topoisomerase II is also necessary for maintenance of genome stability, particularly in the highly repetitive ribosomal (r)DNA gene cluster.

Have the analyses of BLM homologues told us anything about the molecular basis of the defect in Bloom's syndrome cells? The *E. coli* RecQ protein is a constituent of the RecF genetic recombination pathway in *E. coli* [5]. In a genetic background in which the RecF pathway is operational, *recQ* mutations cause a deficiency in conjugational recombination and hypersensitivity to ultra-violet (UV) light, consistent with a role for RecQ protein in recombinational repair of UV-induced pyrimidine dimers. Although some similarities exist between *recQ* and Bloom's syndrome mutant cells, it is clear that RecQ protein is required for efficient genetic recombination and DNA repair, whereas the phenotype of Bloom's syndrome cell lines is one of genetic hyper-recombination coupled with an apparent proficiency in DNA repair.

BLM and Sgs1p may be true functional homologues, as the phenotype of an *sgs1Δ* strain includes hyper-recombination and a high level of genomic instability [6,7], closely resembling that of a Bloom's syndrome cell line. The hyper-recombination in *sgs1Δ* strains is manifested as an increase both in mitotic excision recombination and in inter-chromosomal homologous recombination ([6] and our unpublished data). The observed hyper-recombination is at least partially independent of the *RAD52* and *RAD1* genes, suggesting that Sgs1p is not a general component of one of the two well-characterized yeast recombination pathways. In *sgs1* mutant strains, the rate of missegregation of chromosomes is increased in both meiosis and mitosis [7], consistent with Sgs1p having a cellular role in conjunction with topoisomerases. The defect in *sgs1* mutants primarily causes mitotic and meiotic chromosome non-disjunction rather than chromosome loss. By analogy, the observed male infertility and female subfertility of Bloom's syndrome patients may result, at least in part, from meiotic non-disjunction.

Although many pieces of the puzzle remain to be fitted into place, studies on Bloom's syndrome cells and yeast

Figure 2



Loss of the BLM helicase may affect recombination efficiency (1), which can indirectly influence DNA replication because of the probable role of the recombination machinery in 'repairing' stalled or damaged replication forks (2). Alternatively, BLM may be required directly in replication (3). A failure to complete recombination or replication could generate entangled DNA (4) and a consequent failure during cell division to disjoin sister chromatids at mitosis or homologous chromosomes at meiosis.

sgs1 mutants have suggested mechanisms by which a deficiency in helicase function could lead to genomic instability (Fig. 2). One possibility is that a recombinogenic lesion is generated at an increased frequency in *sgs1* and *BLM* mutant cells, and that this leads to hyper-recombination and, indirectly, to chromosome non-disjunction. Alternatively, the helicase may act either directly in replication or in a recombinational repair process specifically aimed at restoring replication to regions of the genome containing stalled or damaged replication forks. In either case, a failure to complete replication, because of a *sgs1*/*BLM* mutation, would result in non-disjunction arising through the generation of unresolvable replication intermediates.

Sgs1p might act at a late stage in DNA replication alongside its partner topoisomerase II. We have previously postulated [7] that Sgs1p is involved in unwinding the small fraction of DNA located at sites of converging replication forks that cannot be replicated conventionally because of torsional or steric constraints. Unwinding of these replication intermediates is a source of the intertwinings between sister chromatids which must be resolved by a topoisomerase prior to sister-chromatid separation at anaphase. The efficiency of this overall process will thus be a major determinant of the fidelity of chromosome segregation during cell division.

A lot remains to be clarified regarding the molecular mechanisms underlying cancer susceptibility in Bloom's syndrome and normal individuals. However, it is hoped that further studies, both in Bloom's syndrome and yeast cells, will help to reveal the exact mechanism by which the BLM family of helicases maintain the integrity of the eukaryotic genome and consequently suppress cancer.

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